REMARKS

Applicant's counsel thanks the Examiner for the careful consideration given the application. Claims 2, 6, 14-17 have been cancelled in order to overcome the Examiner's objections. The remaining claims have been amended in order to more clearly define the invention; no new matter has been added.

In response to paragraph 4 on page 2 of the Office action, applicant is enclosing an English translation of the certified copy of the foreign priority application No. MI2003A000860 filed on April 29, 2003; the translation of the certified copy is accurate.

In the most recent Office action, the Examiner states that the present Application is obvious in view of Sun *et al.* and Cutrona *et al.* These prior art documents are briefly summarized as follows.

The research purpose of Sun *et al.* was to develop "*a different and more tumor specific approach*" using PNA molecule (see page 1558 first paragraph) by conjugating PNAs with analogs of hypothalamic peptide somatostatin. Said conjunction allows improved PNA cellular uptake only in cells expressing somatostatin receptors (especially tumor cells expressing said receptors), since somatostatin and its octapeptide agonist analogs are rapidly internalized after binding to type 2 receptor (see page 1558 first paragraph).

To evaluate experimentally PNA-somatostatin analog conjunction, Sun *et al.* targeted *N-myc* oncogene belonging to the same family as *c-myc, l-myc* and *v-myc* and playing a key role in the control of normal cell proliferation, differentiation, and tumor genesis and progression (see second paragraph at page 1558).

The targeting strategy involves **anti-sense** PNAs conjunction to somatostatin analogs and their cellular administration to block m-RNA translation and thus *N-myc* expression.

The results have demonstrated that somatostatin agonist analog conjugation to PNAs accounts for a better cellular uptake of conjugated molecules. Nevertheless, gene transcription impairment, by using anti-sense PNAs conjugated to somatostatin analogs, depends on cell target. Indeed, *N-myc* m-RNA transcription was impaired in neuroblastoma IMR32 cells

(expressing somatostatin receptor). At the same time, this paper shows that GH3 cells, expressing both *N-myc* gene and somatostatin receptors, do not show any effect on N-*myc* transcription.

In view of the above it is evident that Sun *et al.* efforts were focused, essentially, on cell PNA delivery optimization. The solution that Sun proposed was the conjunction of PNAs with somatostatin analogs to ameliorate cell membrane crossing.

Regarding Cutrona *et al.*, the purpose of this study was to explore the conditions that would make anti-gene PNAs effective in intact cells *in vitro* (see preamble of the article at page 300 first paragraph). In particular, this study elucidates the influence of Nuclear Localization Signal (NLS) peptides conjunction to PNAs (above all PKKKRKV peptide) in cell nuclei penetration (see page 300 second column).

To address this issue Cutrona *et al.* designed a *c-myc* anti-gene PNA complementary to unique sequence in the second exon of *c-myc*. This molecule was administered to Burkitt's lymphoma cell lines characterized by *c-myc* hyper-expression resulting from chromosomal translocation (see preamble of the article at page 300 first paragraph).

Cutrona *et al.* demonstrated, by using this experimental approach, that anti-gene PNAs specific for a gene, for example specific for *c-myc* gene, and fused to NLS peptides are localized into the nucleus and are able to block the expression of the targeted gene (for example *c-myc*) and, thus, to inhibit its biological functions.

In view of the above reasoning, it is evident that also Cutrona *et al.* attempts were focused on cell PNA delivery optimization, with particular reference to PNA delivery into intact cells. The solution they proposed was the fusion of PNAs with NLS (in particular with PKKKRKV peptide) to improve cell uptake.

In conclusion, the scope of the cited prior art documents is different with respect to the purpose of the pending Application that regards sense (anti-gene) PNAs molecules directed against *N-myc* gene for treating those pathologies caused by *N-myc* over-expression, such as neuroblastoma, medulloblastoma, rhabdomiosarcoma etc.

Moreover, applicant submits again that Sun *et al.* refers to **anti-sense** PNAs complementary to sense strand of human *N-myc* gene, while the claimed molecules are **sense** anti-gene PNAs complementary to anti-sense strand of *N-myc* gene.

A person of ordinary skill in the art knows that the mechanism of action of the two molecules are completely different because sense anti-gene PNAs target the gene (transcription and translation), instead anti-sense PNAs target mRNA (translation). Looking at these relevant differences, it is clear that the prior art references do not render obvious the improved down-regulation of *N-myc* expression obtained in the present invention using sense anti-gene PNAs with respect to the use of the anti-sense PNAs.

Concerning Cutrona *et al.*, applicant asserts that the cellular model used, to optimize PNA delivery protocol into intact cells, was derived from **Burkitt lymphoma**.

This cell line is characterized by *c-myc* over-expression caused by its translocation from chromosome 8 to chromosome 14, often close to the enhancer of the immunoglobulin heavy chain locus (IgH). The **neuroblastoma** cell lines (and also the other cell lines analyzed), used in the present invention, instead, are characterized by *N-myc* over-expression caused by N-myc DNA amplification from 3 to 1500 copies.

In view of the last consideration, the following differences are evident:

1) The referring genes of the two cellular models above reported are *c-myc* and *N-myc*. These are not **IDENTICAL** genes; instead they are **RELATED** genes belonging to the same family.

2) The up-regulation of *myc* gene in the two above disclosed cell lines is caused by two completely different mechanisms.

In particular, the cellular models disclosed in the present invention show an increase of N-myc gene copy number, compared to the cellular model used by Cutrona *et al.*, where *myc* up-regulation is a consequence of the gene translocation under IgH enhancer.

In view of the observations above reported, it is clear that the administration of a sense anti-gene PNA of the invention in the cell lines of the present Application and in the cell

line used by Cutrona *et al.*, would provide results significantly different among the two cellular context with regard to *myc* gene down-regulation, and therefore unpredictable for a person of ordinary skill in the art.

Moreover, even if Cutrona speculates that anti-gene PNAs, showing low toxicity when administered both in cultured and *in vivo* cells, could lead to exciting new therapeutic approaches (see page 302 second column), applicant stresses the physicochemical and biological intrinsic difficulties of these "unspecified" therapeutic approaches.

As said before, PNAs are very appealing molecules as DNA mimics. Their high biological stability with strong-binding affinity to complementary DNA and/or RNA is well known. Nevertheless, several limitations have to be taken into account using these molecules in clinical trial. Above all, PNAs have low aqueous solubility, their DNA binding orientation is ambiguous, and their membrane permeability is poor.

PNAs research, like any other pharmaceutical research, is highly unpredictable. For example, Sun *et al.* also demonstrated that by administering the same anti-sense PNA molecule complementary to *N-myc* mRNA, the gene expression impairment depends on the specific cell line used (applicant reminds that GH3 cells, expressing *N-myc* and somatostatin receptors did not show *N-myc* down–regulation by applying Sun *et al.* experimental conditions).

Therefore, the teachings of Sun *et al.* would not have been sufficient to guide the person of ordinary skill in the art to the solution of the present invention.

ITEM 6 - RESPONSE TO ARGUMENTS

Regarding the Examiner's objection that the instant specification shows that one particular sense molecule inhibited better than one particular anti-sense molecule (thus the results in the present Application are not commensurate in scope with what is claimed), applicant is submitting herewith a Declaration of Mr. Roberto Tonelli, one of the inventors of the present Application. The Examiner is also informed that, through a series of assignments which are not yet recorded, the present patent application is owned by Biogenera, S.r.L., and Biogenera S.r.L. is owned by Roberto Tonelli (who signed the enclosed Declaration) and by Andrea Pession, another co-inventor of the present patent application.

This new experimental evidence regards the ability of further sense (anti-*N-myc*) PNA molecules to down-regulate *N-myc* expression. In particular, the Applicant has tested 4 sense anti-*N-myc* PNAs (named PNA-1, PNA-2, PNA-3 and PNA-4) falling in the general definition of claim 1 as now presented.

The impairment of *N-myc* gene expression induced by said sense anti-*N-myc* PNAs has been assessed by *N-myc* mRNA quantization by real-time PCR analysis; while the biological effects of sense anti-*N-myc* PNAs administration has been evaluated by cell growth inhibition assays.

The results clearly demonstrate that all sense anti-*N-myc* PNAs tested when administered to neuroblastoma Kelly cells (over-expressing *N-myc* gene) induce a down-regulation of *N-myc* mRNA transcription (see figure 1 of Declaration). Moreover, said cells do not proliferate as usual (see figure 2 of Declaration).

On the contrary, normal fibroblast cells, named Phoenix and not over-expressing *N-myc* gene, do not show any impairment of both *N-myc* mRNA transcription and cell growth (see figures 3, 4 of the Declaration).

These results demonstrate that claim 1 is commensurate in scope and that other PNAs falling under the definition of claim 1 show good results in terms of down-regulation of *N-myc* expression.

In view of all the foregoing, it is believed that the claims are now in condition for allowance, which is respectfully requested. If any further fees are required by this communication, please charge such fees to our Deposit Account No. 16-0820, Order No. BUG5-38919.

Respectfully submitted,
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